THE DYNAMIC FACTOR IN REGENERATION.

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With the publication of the data here presented the series of experiments that I have carried out on *Tubularia* for several years may be considered as temporarily brought to a close. I take this opportunity therefore to sum up the evidence bearing on the problem of the formative factors of regeneration, as exhibited by this hydroid. In the course of my experiments tentative hypotheses have been proposed here and there that have at least served to suggest further experiments. The conflicting evidence sometimes inclined me towards one point of view, sometimes towards another; yet, all in all, the same general line of thought, if sometimes vague, can be traced through the attempts to analyze the results. It will be my endeavor here to bring more into the foreground those theoretical deductions that seem to me at present to be best in harmony with the experimental evidence.

That the dynamic factor in regeneration is not primarily the outcome of physiological movements of the animal, or of its parts, is made probable not only by many facts familiar to every student of regeneration, facts that show that the new part often develops under conditions where movement or function in this sense is absent, but also by the important experiments of Zeleny and of Stockard with the jelly-fish *Cassiope*. They have shown that when one half of the disc is brought to rest by removing the sense organs (and scratching a barrier zone across the connecting ectoderm) the quiescent half regenerates as well and as rapidly as the half left pulsating during the period of regeneration.

Since every part of the stem of *Tubularia* is capable of producing a hydranth the inhibition of basal development is obviously due to the presence of a hydranth or of a developing hydranth at the oral end. Two alternative chemico-materialistic explanations have been suggested for cases like this. (*A*) The oral hydranth may use up some materials necessary for the formation

of a basal hydranth. (*B*) The hydranth may produce some materials that inhibit the development of other hydranths from the remainder of the piece; hence the inhibition, as long as a hydranth is present or developing. On first thought these alternatives would seem to cover the only possible ways in which the problem of regeneration may be presented — at least as long as the problem is confined to purely physiological actions of a chemical order. There are, however, not a few considerations indicating that the fundamental interpretation may lie in a different conception of the problem. I shall try here to emphasize this other point of view without attempting to develop it into a theory of regeneration. At most we may hope at the present time to find in the facts some indication of the nature of the problem if not its entire elucidation.

A number of experiments have been made that seem to indicate that the temporary inhibition of the development of the basal hydranth in *Tubularia* is not the result either of the using up of materials by the oral hydranth, or of the setting free of inhibitory stuff. The simultaneous development of hydranths at both ends of a piece, which frequently occurs in short pieces, is a case in point. Both ends develop at the same rate as when a single hydranth develops, and not half as fast as the hypothesis demands. Again the development of a basal hydranth does not appear to inhibit the oral development as we should expect if the result were dependent simply on the presence of materials in the stem. Some experiments of MacCallum's with plants have an important bearing on this point. If the terminal bud of the bean is removed, the buds in the axils of the cotyledons develop. But if the activity of the terminal bud is simply lessened by inclosing that part in an atmosphere of hydrogen, the basal buds do not develop. Hence the result is due not to activity of the terminal end, but to its presence or absence. In a different way the same fact is brought out. A piece of willow stem is cut off, its middle third is inclosed in a tube filled with moist air, so that the buds in this part are encouraged to begin their development; the dry air retarding the development of those outside. After the middle buds have unfolded, the entire piece is inclosed in a moist chamber, when the more apical buds sprout forth, while none

of the buds basal to the middle region develop. The presence of growing shoots in the middle of the piece does not inhibit the apical buds from developing, if external conditions are supplied favorable to their growth, but the basal buds are inhibited by the presence of shoots on the more distal parts. These facts are incompatible with the assumption that the results are due to the presence of materials used up by those parts that develop first to the exclusion of other parts. They also show that the alternative view is untenable, for, the presence of growing shoots in the middle of the piece is not antagonistic to the development of shoots in other regions provided those regions are situated more distally.

In the case of *Tubularia*, it is more difficult to present convincing evidence that distal hydranths do not produce materials inhibiting the development of basal hydranths, improbable as such an interpretation may now seem. But the fact that basal hydranths do develop after the oral hydranths have formed may seem to discredit this view. Here, however, an apparent paradox is found. The experiments seem to show that when the oral hydranths develop, the basal hydranths are retarded in development, but they do develop later, and the results also show that if both start simultaneously both develop at the normal rate. The paradox is due, I think, to two antagonistic factors at work at the same time. Admitting that the oral development tends to inhibit the beginning of basal development, we also find that if other influences suffice to start both simultaneously, the on-rush, so to speak, of the process once begun changes the conditions that tended to prevent the starting. Strange as this seems it is little more than a statement of the facts. The same results may be put in a somewhat different way. A cut end being present, whether oral or basal — the conditions that call forth hydranth formation are given. Experiments show that the oral end tends to develop first, its development acts as a partial inhibition of the basal hydranth-formation. If this influence is strong enough the basal development is temporarily held in abeyance, but if not the inhibition is overcome. Once overcome, the formative influences do not check the further action of the basal end. In this connection it is curious to note that small oral pieces produce simultaneous hydranths more often than larger

pieces. The interpretation of this seems to be that the tendency to produce hydranths, both oral and basal, is stronger near the distal end and decreases basally. In short pieces the sensitiveness of the two ends to those influences that call forth the hydranth is so great that both ends develop simultaneously or nearly so, hence the oral end has not time to get a sufficient start over the basal to stop its development. It should be noted in passing that it is probable that the influence preventing basal development is not only the oral development, but a direction-factor present in the stem at all times. This factor we call polarity. The interesting point is that this factor seems to be more capable of inhibiting basal hydranth formation when an oral end is developing than when such development has not yet begun. The basal development, however, does not appear to delay the oral process. It is acting against the polarization and its influence is less felt throughout the stem, as experiments by Stevens and myself have shown. These considerations lead, I think, to the view that the essence of our problem lies in that peculiarity of the piece that we designate its polarity, and not in the absence or presence of formative substances in Sach's sense.

If our analysis is correct, we are led to look upon living material as possessed of a certain formative principle that has so to speak a "sense of direction." The next step will be to study the nature of this principle and see what properties we are justified in ascribing to it; for while it may be beyond our powers at present to state precisely the nature of the directive principle, we may at least be enabled to work out its manifestations. Some of these manifestations become apparent in the study of the regeneration of *Tubularia*. One of its most striking modes of action is seen in the inhibition of basal-hydranth formation. Most interesting is the result that its action becomes intensified by developmental processes going on at the oral end, as shown by the fact that if the oral development is suppressed by tying that end, the basal development is much accelerated. It is accelerated in the sense that basal hydranths more often develop at once than when both ends are open, but not in the sense that the basal development is faster than when this end also gets as early a start as the oral end. In other words, there is no speed-

ing up of hydranth formation as such, but the initial inhibition is overcome.

The special problem with which this paper deals is the nature of what takes place at the basal ends when the oral end is kept open and when it is tied. Is the retardation of such a kind that a slower process of development is going on at the basal end while the oral end is developing, or does the basal end not really begin to develop until the oral end has formed its polyps. If so, what gives it its start later? The following experiments were devised to study these questions.

Experiment I. — The purpose of the experiment was to determine whether when both oral and basal ends of a piece are left open constructive changes are slowly going on at the basal end. Some pieces were cut off and left open (*A*); later other pieces were cut off and the oral ends tied (*B*) and at the same time the oral ends of (*A*) were tied. It was found that the basal ends of the (*A*) pieces did not develop faster than those of the (*B*) pieces, showing that the changes at the basal end of (*A*) are not progressing, but are held in check by the developing oral hydranth.

Control I. — In some pieces the old hydranths were left intact and the pieces cut off. No basal hydranths began to develop until the old heads began to be absorbed. The presence of the old heads inhibited the development of the basal hydranths until the heads had degenerated when the latter appeared.

Experiment II. — In order to find out whether, when the oral end is tied, changes take place throughout the piece that tend to make more rapid the development of basal hydranths, or whether these changes are localized at the basal end where the new hydranth develops, the following experiment was tried. The oral ends of many pieces of the same length were tied. Then after several hours' interval differing in several experiments, the basal end was cut off, (*a*) just inside of the area that would form the basal hydranth, (*b*) in the middle of the piece, (*c*) just below the ligature. In general the development of the basal hydranth was delayed as compared with control pieces tied but not cut off at the basal end; the delay was the greater the further removed the cut from the basal end, despite the fact that oral levels tend to regenerate faster than more basal levels. The differences

are more apparent the longer the time that elapses before the basal pieces are removed. The differences are not very marked at the different levels indicating perhaps that changes take place throughout the piece and not only at the basal end although more pronounced in the latter. The different levels of the cuts make it difficult to ascribe the results solely to the general changes in the piece, for the more orally situated cut ends have an advantage in level as other experiments have shown.

Experiment III. — Pieces were cut off at the same oral levels. After 23 hours the hydranth region at the oral end was cut off of some pieces (*A*), others were cut in two in the middle of the piece (*B*), and for a control some pieces were left as before (*C*). A slight retardation occurred after another 12 hours in (*A*), less in (*B*) as compared with (*C*). Removal of the hydranth forming region after 23 hours causes delay but the delay is not so much as though a new hydranth had developed at the new cut, showing that changes directed towards hydranth formation are going on not only in the region where the hydranth will develop but at more basal levels as well.

Experiment IV. — This experiment was like the last, except that the basal ends of all the pieces were tied, thus preventing the basal end from exerting any influence on the result. Other experiments had shown, however, that the basal development, even if it occurs, has apparently no retarding influence on the oral development. The results, as was to be expected, were the same as in the last experiment. It is interesting to note that in both the influence of the cutting causes a greater delay in the first appearance of the primordia of the hydranth than on their later development; for later the differences seem to be less than at first. This may be due to an acceleration extending throughout the whole time that is more effective after a beginning has been made than before the start.

Experiment V. — Some previous experiments had left undecided the question whether, when the oral end is left open for several hours and is then tied off, the basal development is more rapid than when the oral end is tied at once. If such an acceleration really occurs it might seem to indicate that changes take place in the oral end that produce accelerating materials even for the

basal ends. I was particularly anxious to settle this point definitely, for obviously, if such acceleration could be proved, it would furnish evidence in favor of a chemical process, especially since other experiments had seemed to show that the basal end does not begin its development when both ends are left open. I have carried out rather an extensive series of experiments that give, I think, a definite answer to the question.

When pieces are left open at both ends from four to nine hours, and are then tied at the oral end, the basal development is slightly retarded as compared with its development in pieces tied at once. There is little evidence in favor of the view that the later tied pieces can make good the loss of four to nine hours, and of course they can not catch up if a longer time elapses. Whether they may do so in later stages is more difficult to decide, but this does not concern the main point here raised.

Individual differences in rate, differences in stems, and uncontrollable differences in level tend to obscure results that depend on only four, six, and nine hours differences in start. The above statement holds, therefore, only for average results. There was found no evidence in favor of actual acceleration, whether there is some relative acceleration is difficult to decide. If the hydranths do not develop promptly *i. e.*, if a long time elapses between the tying and the appearance of hydranths, the initial differences of a few hours may be lost.

Experiment VI. — Another attempt was made to see whether changes take place in the piece as a whole, after it is cut off, that make more rapid the development of an *oral* hydranth when a new cut is made.

Pieces were removed and after four hours somewhat more than the oral hydranth region was cut off. In some cases the newly cut ends developed as fast as did the hydranth in the small pieces cut off, but the latter may have been retarded by the operation or by the smallness of the pieces; yet in some cases the development of the newly cut ends was as rapid as in control uncut pieces. This result indicates that changes take place in the pieces behind the actual region of hydranth formation that lead toward the development of a hydranth.

Experiment VII. — In this case pieces first cut off were after

ten hours cut in two in the middle. Comparing the rates of development of the oral ends of the oral halves with that of the oral ends of the basal halves it appears that the latter are slower, but there is evidence that the retardation may be somewhat less than the ten hours difference in initial start. The basal halves in this experiment are also somewhat behind the basal halves in the last experiment, which seems to show that the general changes in the excised pieces that go towards oral development decrease from the cut ends inwards.

It has not seemed necessary to give the details on which these general conclusions are based. The nature of the case makes it difficult to obtain results as definite as one might wish, despite the precautions that were undertaken to make the conditions as uniform as possible. The general conclusion that changes take place in the piece as a whole, after its removal, that are in the direction of hydranth formation, seems fairly certain. Less certain perhaps is the evidence to show that when the oral end is tied similar changes take place in the pieces that accelerate basal development in regions beyond the hydranth forming region, but this conclusion too is, I think, quite probable. The nature of these changes is not revealed.

The Dynamic Factor in Egg-Development.

Students of the processes of regeneration have without exception made use of the term polarity to express a directive factor observable in their results, and to this factor is sometimes ascribed an active rôle as a controlling influence, at other times the term is used descriptively merely as a statement that the new structures are directed in the same way as the part removed. In both respects the word has been useful, however vague our conception of what polarity may be. Our analysis of the process has now gone sufficiently far, I think, to justify us in an attempt to come to closer quarters with the term. Without reviewing the opinions that have been expressed as to the nature of polarity, I shall try to contrast two views of its nature that seem to me to represent the two main lines that speculative thought has followed. It should be noted that the term is used equally by students of embryology and by students of regeneration. The former

finds axial relations in the egg — polarity, bilaterality, radial symmetry, etc.; the latter finds the new organs regenerated in definite relations to the old. To some observers the distribution or the stratification of the materials of the egg, has seemed a sufficient basis for the results referred to under the term polarity; to others it has seemed more probable that there exists in the egg an arrangement or structure that has axial relations from which result not only the depositions of the formed materials but also the nature of the action of the parts. Polarity is from this latter point of view not simply a passive structure, but a relation of the parts that directs the shifting series of changes that we call development. At one time one of these views has seemed more probable; at other times the other. The history of modern experimental embryology and regeneration shows the influence that these views have had on those who have followed the new work. In a general way the two views may be classed as the materialistic or chemical and the dynamic or physical conceptions of the developmental process. At present it seems to the writer that the evidence has been steadily pointing to the second of these contrasting views as the more probable. As far as the egg is concerned, the recent experimental work goes to show that the visible inclusions of the protoplasm (yolk, oil and other granules perhaps) are not the fundamental causes of the formative processes, although they may be needed in certain regions to carry out the future development of the structures that there appear. In regard to regeneration it has been evident for some time, that the specification or the differentiation (with its concomitant products), cannot be unreservedly utilized as a basis for an explanation of formative processes that take place. For example, if the gross materials or the differentiations of the head end of a planarian are the causes of that region being a head, it is inexplicable that when the head is removed it could regenerate a tail. There must be something else behind what we see that is responsible for the change that takes place. These and other considerations lead to the view that there exists a fundamental property of living matter that is the formative principle of development. On two former occasions, when attempting to analyze the results of regeneration in *Tubularia*, the author tried to account for the re-

sults of polarity on the basis of a stratification of the materials. Influenced at the time by recent results in experimental embryology that seemed to show that visible substances of different kinds in the egg are really responsible for the development of its parts, the same idea was applied to the problem of regeneration, despite the fact that I had on more than one occasion rejected the hypothesis of formative stuffs, in Sach's sense, as sufficient to account for the facts of regeneration. Yet a careful reading of the papers here referred to will show that I still held, though perhaps not always consistently, to the conception that back of these differentiated materials lay the real differentiating factors.[1] It now seems to me that the evidence, which at that time seemed so strongly to favor the idea of the importance of the grosser materials of the egg, is insufficient to establish its case, and that the important factors of development are dynamic properties of the bioplasm, rather than the formed products of the egg, or of the differentiated products of the adult animal. This statement does not mean that the visible products in the egg play no rôle in development. The evidence still shows that they may do so, but their rôle seems to be secondary, not primary.

The interrelation of the parts seems to be one of the most evident expressions of the fundamental formative influences. Several years ago a consideration of a number of results in regeneration led me to state that this relation might be expressed as a sort of tension. This view has been objected to on the ground that it does not appear to explain the matter any better than before. In a moment of doubt and in order to give the

[1] One further word of explanation. The rate of hydranth formation varies with the distance of the cut end from the original hydranth. I have spoken of this difference in rate as explicable on the assumption of the hydranth-forming materials decreasing toward the base, *i. e.*, away from the hydranth. It was unfortunate to have used the term hydranth materials, although I made sufficiently clear in the text that I did not mean to invoke the stuff-hypothesis in this connection. It is not entirely clear on what the difference in rate depends; most probably on the stem being less specialized as a store-house of food substance nearer the hydranth; probably also on some difference connected with the thickness of the walls with which the specialization may also be connected; possibly neither of these but some more fundamental characteristic is responsible for differences in rate. In any case it is not obvious that there is any connection between this difference in rate and the polarity of the piece. The latter is the same for all levels — the time it takes the piece to be remodelled seems to be referable to something else.

statement a meaning for those who believe no suggestion to be of value unless it refer the problem to ordinary properties of inorganic bodies, I suggested that osmotic pressure might be the cause of the tension differences in the parts. This was an unnecessary concession. The behavior of fluid crystals (according to Lehmann) shows that the formative changes can be accounted for on the basis of a tension exhibited by the molecules of the substance of the crystal. While the organism may not be put down as a fluid crystal, still we see that physical properties other than osmotic pressure and surface tension may play an all-important rôle in form-changes. It may be that a similar property is the cause of the formative changes in the organism. In any case, the facts that I had in mind suggested that tension of some sort is an important dynamic factor in development, perhaps *the* important factor. The facts still seem to me to indicate some such relation between the parts, and no one regrets more than I that we cannot "explain" the results even if my suggestion prove to be in the right direction.

Still later a consideration of certain facts of development led to the suggestion that two known properties of the organism — contractility and irritability — also play a very important rôle in embryonic and regenerative development. I shall not attempt to review here the argument which led to this point of view. How far and in what sense contractility and irritability are better expressions of the tension hypothesis, it is not easy to state. So far as contractility is concerned Lehmann's recent important paper on "Scheinbarlebende Kristalle"[1] shows the possibility at least of referring this property also to a condition of molecular tension. We are still too ignorant of the physical basis of irritability to make speculations in this direction profitable, but it may be well not to lose sight of this property of living matter in our attempts to analyze further the problem of development.

Stereometry of the Bioplasm.

Polarity implies difference in one direction. Every student of regeneration knows that in all three dimensions of space the same factor is present. Polarity is therefore only a part of the problem,

[1] *Biol. Centralb.*, XXVIII., 1908.

and so far as it draws attention away from the whole problem it seems best to substitute the term stereometry.

Sufficient evidence has accumulated, I think, to show that stereometry has a dynamic side — in so far as it is a result of the molecular factors that determine the relations of the parts to each other. A question of fundamental importance here presents itself. If the formed substances at each level are the products of the bioplasm, must not the bioplasm itself be stratified in nearly the same sense? It was this idea that I had in mind when I wrote in 1906: "If we imagine a stereometric network as a part of the specialized structure, we must be prepared to admit that it changes at each level as the structure changes. Therefore it seems to me simpler to base our hypothesis of polarity on the difference in differentiation itself, and not on an imaginary polarized system associated with the living materials." But the point I overlooked was that there is no need to suppose that a heterogeneous network of bioplasm exists because the visible structure formed by it is different. The relation of the polarized material to the ends of the material (indeed to all its directions) suffices to account for the difference of level. In fact if the stereometry rests on a dynamic and not a statical relation of the parts this is the logical standpoint.

It has been suggested that irritability may be related to the dynamic factor of development.

The effects of irritability at any level may be realized through the chemical changes inaugurated. These chemical changes once started may, if enzymatic, thenceforward continue (*unless checked by other chemical processes*), *independently of the factor that set them going.*